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Corticosterone regulates multiple colour traits in *Lacerta* [*Zootoca*] *vivipara* males

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Abstract

Ornamental colours usually evolve as honest signals of quality, which is supported by the fact that they frequently depend on individual condition. It has generally been suggested that some, but not all types of ornamental colours are condition dependent, indicating that different evolutionary mechanisms underlie the evolution of multiple types of ornamental colours even when these are exhibited by the same species. Stress hormones, which negatively affect condition, have been shown to affect colour traits based on different pigments and structures, suggesting that they mediate condition dependence of multiple ornament types both among and within individuals. However, studies investigating effects of stress hormones on different ornament types within individuals are lacking, and thus, evidence for this hypothesis is scant. Here, we investigated whether corticosterone mediates condition dependence of multiple ornaments by manipulating corticosterone levels and body condition (via food availability) using a two-factorial design and by assessing their effect on multiple colour traits in male common lizards. Corticosterone negatively affected ventral melanin- and carotenoid-based coloration, whereas food availability did not affect coloration, despite its significant effect on body condition. The corticosterone effect on melanin- and carotenoid-based coloration demonstrates the condition dependence of both ornaments. Moreover, corticosterone affected ventral coloration and had no effect on the nonsexually selected dorsal coloration, showing specific effects of corticosterone on ornamental ventral colours. This suggests that corticosterone simultaneously mediates condition dependence of multiple colour traits and that it therefore accounts for covariation among them, which may influence their evolution via correlational selection.

Introduction

In many animal species, the expression of conspicuous ornamental colours used as signals in intra- and intersexual reproductive interactions depends on condition (Velando *et al.*, 2006; Kemp & Rutowski, 2007; Peters *et al.*, 2007, 2008; Hill, 2011), which is generally defined as an index of individual viability that relates to nutritional status and health (Anderson, 1994). Current theory states that only individuals in good condition can afford the costs associated with development, maintenance or display of exaggerated coloration and, thus, that these colour traits evolved as honest indicators of individual quality (Johnstone, 1995). Studies that simultaneously investigated the function of multiple ornament types in the same species, suggested that carotenoid-based and (although studied to a lesser extent) structural colours, but not melanin-based colours, are condition-dependent traits, that is, depend on body condition (Senar *et al.*, 2003), food availability (Kogel & Prijs, 1996; Hill, 2000; McGraw *et al.*, 2002)

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or parasite load (Hill & Brawner, 1998; McGraw & Hill, 2000). The fact that no congruent effects among ornament types were found in studies investigating several ornament types at the same time led to the view that colours produced by different pigments and/or structural components are developmentally and/or functionally independent (Candolin, 2003) and that, whereas condition dependence drives the evolution of some types of ornaments, other ornaments are governed by alternative evolutionary pathways (Badyaev & Hill, 2000).

However, this idea has been challenged by studies showing that melanin-based coloration also correlates with certain measures of individual condition (Fitze & Richner, 2002; Parker *et al.*, 2003; review in McGraw, 2008). These studies support that melanin-based traits can also function as condition-dependent traits and, therefore, that different types of colours might share common evolutionary mechanisms. In this regard, recent studies investigating single ornaments of different species found that corticosterone levels (the main glucocorticoid released during stressful conditions in vertebrates; Wingfield *et al.*, 1998) are linked to the expression of different types of colorations (Calisi & Hews, 2007; Loiseau *et al.*, 2008; Roulin *et al.*, 2008; Fitze *et al.*, 2009; Lobato *et al.*, 2010; Grindstaff *et al.*, 2012; Martínez-Padilla *et al.*, 2013). This suggests that stress hormones, which negatively affect condition in situations of long-term stress (Moore & Miller, 1984; Tokarz, 1987; Dhabhar & McEwen, 1997; Cote *et al.*, 2010), may account for condition dependence of multiple types of ornaments in the same individual. However, to our knowledge, no study exists that investigated whether corticosterone accounts for the condition dependence of multiple types of ornamental traits in the same individual, and thus, no evidence exists that stress hormones or corticosterone is general mediator of condition dependence. Unravelling whether multiple colour traits of the same species are condition dependent is crucial for the determination of shared common regulatory mechanisms of condition dependence, which helps to understand the origin of covariation among ornamental traits and their coevolution (Endler, 1995).

Here, we investigated the determinants of condition dependence of melanin- and carotenoid-based coloration in male common lizards (*Lacerta* [*Zootoca*] *vivipara* Jacquin 1787). We manipulated blood corticosterone levels and body condition (via food availability) using a crossed two-factorial design and measured their effect on melanin- and carotenoid-based ventral coloration. This design allowed testing condition dependence of multiple ornament types if mechanisms of condition dependence are shared or exclusive among ornament types. Previous studies showed that the common lizard's carotenoid-based coloration is affected by corticosterone (Fitze *et al.*, 2009; San-Jose *et al.*, 2013) and that its effect depends on food intake (Cote *et al.*, 2010). However, it is unknown whether corticosterone and food availability also influence the expression of melanin-based spots. Finally, we compared treatment effects on ventral colour traits with those on the dorsal, melanin-based coloration, which is subjected to natural selection (Lepetz *et al.*, 2009). This comparison further allowed determining the specificity of condition dependence on ornamental colour traits (Cotton *et al.*, 2004).

Materials and methods

Species description

The common lizard is a small lacertid that inhabits peatbogs and moist heathlands and that is widely distributed across Europe and Asia (Sindaco & Jeremcenko, 2008). Male common lizards display a conspicuous ventral coloration that consists of melanin-based black spots surrounded by a white-to-orange carotenoid-based coloration (Fitze *et al.*, 2009; San-Jose *et al.*, 2012c). Ventral coloration is concealed to avian predators but visible to conspecifics when lizards push up on their forelegs exposing their throat and part of the belly (Martin *et al.*, 2013). Ventral coloration is also laterally visible given that the outermost longitudinal scale rows of the belly are partly laterally oriented (L. M. San-Jose, personal observation). In common lizards and other reptiles, amphibians and fish, coloration is produced by the combined action of different absorbing and reflecting chromatophore cells that are arranged in adjacent layers to form the dermal chromatophore unit (Bagnara *et al.*, 1968; Grether *et al.*, 2004). In the common lizard, carotenoid-based coloration is generated by the basic dermal chromatophore unit, in which carotenoids are deposited in xanthophores located in the first and outermost dermal layer (Breathnach & Poyntz, 1966; Bryant *et al.*, 1967). The next layer is composed of iridophores, which contain finely arranged packs of purine crystals that produce colour by multilayer interference (Grether *et al.*, 2004). Finally, the third and innermost layer, located beneath the iridophores, is composed of melanin-containing melanophores. Black colour patches are exclusively due to the presence of several layers of melanophores stacked one upon another from the basal layer of the dermis to the epidermis (Kuriyama *et al.*, 2006; Alibardi, 2012).

Ventral melanin-based spots and carotenoid-based coloration are sexually dimorphic. Females present no or very few melanin-based spots and less conspicuous yellow/orange coloration than males (Martin *et al.*, 2013). In the here-studied populations, the great majority of the females present no yellow/orange coloration, that is, they exhibit a white belly (Arribas, 2009). Carotenoid-based coloration influences male mating success (Sinervo *et al.*, 2007; Fitze *et al.*, 2009), and males displaying redder hues have increased mating success (Fitze *et al.*, 2009). Carotenoid-based coloration is linked with male body size and body condition (Fitze *et al.*, 2009; Martin *et al.*, 2013), corticosterone levels (Fitze *et al.*, 2009; Cote *et al.*, 2010; San-Jose *et al.*, 2013), dietary lipids and antioxidants (San-Jose *et al.*, 2012b), oxidative damage (Cote *et al.*, 2010), and social interactions (Cote *et al.*, 2008). The function of ventral melanin-based spots has been less studied but existing evidence indicates that the amount of spots on the lizards' belly is linked with age (Martin *et al.*, 2013), and the blackness of the spots is positively related to body size and bite force (K. Huyghe, L.M. San-Jose, J. Schürch, and P.S. Fitze, *unpublished data*). This suggests that ventral melanin-based spots may reflect male aggressiveness, male competitive ability and/or social status as in other lizard species (Cooper & Greenberg, 1992).

Experimental procedures

In July 2008, 36 adult common lizard males were captured at Somport (Central Pyrenees, Huesca, Spain, 42°47′40″ N, 0°31′33″ W, 1634 m above sea level) and housed in the laboratory at the Instituto Pirenaico de Ecología (Jaca, Huesca, Spain). Lizards were individually housed in terraria equipped with two shelters, a water pond and peat soil as substrate (for further details see San-Jose *et al.*, 2012b) and were fed with a wax moth larva (*Galleria mellonella*) every 4 days and provided with water *ad libitum.* Half of the lizards were randomly assigned to a corticosterone treatment group and the other half to a control group. Corticosterone (C2505, Sigma-Aldrich Quimica SA, Madrid, Spain) was administered in 4.5 μL of sesame oil (1.5 μg of corticosterone μL^{-1} of oil; Gonzalez-Jimena & Fitze, 2012) and applied to the back of the lizards every evening for 30 days. Control lizards were treated with 4.5 μL of sesame oil only. This corticosterone dose has been previously shown to double plasma corticosterone levels in the common lizard (Gonzalez-Jimena & Fitze, 2012), and it leads to an increase in corticosterone levels that it is 5% higher than the maximum values observed in the field (Fitze *et al.*, 2009). The applied corticosterone dose thus resembles a representative physiological dose. Significant elevation of blood corticosterone levels has been observed during all throughout the ectopic administration (Meylan *et al.*, 2003), discarding that corticosterone levels decrease with time owing to a downregulation of endogenous corticosterone secretion or an increase in the corticosterone clearance rate as suggested in other species (Goutte *et al.*, 2010).

Using a crossed design, half of the lizards of the corticosterone and control groups were assigned to a high-fed group and the remainder to a low-fed group, which were fed every 4 and 8 days, respectively. Between 12:00 and 13:00 h, one larva was carefully offered to each lizard using a tweezers. When a lizard refused to eat, the larva was left in the terraria and 24 h later, the presence of the larva was checked to determine food consumption. Noneaten larvae were removed. Appetite was defined as the number of larvae eaten relative to the total number of offered larvae. Lizard body mass and body size (snout-to-vent length, SVL) were measured to the nearest 1 mg and 1 mm, respectively.

Colour measurements

Standardized photographs of the belly and back of each animal were taken 1 day before starting the experiment and the day after finishing the experiment as previously described (San-Jose *et al.*, 2012a). Lizards were gently placed into a foam-filled box and covered with a photographic filter lens (Hoya UV-filter, Kenko Tokina Co., Ltd., Tokyo, Japan). This box was placed in a fixed position inside a larger opaque camera box. The distance between the box and the digital camera (Nikon D70S, 6.10 megapixels, with a 105 mm f/2.8 Nikkor objective, Nikon Corporation, Tokyo, Japan) was set at 40 cm. Light was provided by two flashes (Nikon SB-600, Nikon Corporation) positioned at either side of the camera box at an angle of 13° relative to the optical axis.

To measure the coloration of melanin-based spots, four black spots were selected in a fixed region, both on the back and on the belly (dorsal coloration was measured in areas not treated with oil). Using ADOBE PHOTOSHOP CS2 (Adobe Systems, Inc, San Jose, CA, USA), an area of 8 × 8 pixels was selected at the centre of each spot. As for melanin-based coloration, carotenoid-based coloration was measured over an area of 8 × 8 pixels on 4 standard ventral scales. Average red–green–blue (RGB) values were recorded and used to calculate hue (°, in common lizards, high values indicate less red colorations), saturation (%, high values indicate purer coloration) and brightness of each area (%, high values indicate brighter and more intense colorations) (Foley & Van Dam, 1982; Fitze & Richner, 2002). Given that melanin-based traits of common lizards vary more in purity (saturation: mean ± SD = 45.54% ± 15.29) than in brightness (mean ± SD= 14.54% ± 2.77, Levene's test for equality of variances: $F_{1,64} = 31.85$, $P < 0.001$), we decided not to use brightness as a mere equivalent of blackness as previously performed in some studies (McGraw & Hill, 2000). Instead, we calculated blackness (100% completely black, 0% completely white) from average red–green–blue (RGB) values using the following formula: $(255 - [0.33 \times R - 0.59 \times G - 0.11 \times B])/2.55$ (Palus, 1998; Muck & Goymann, 2011), which accounts for variation in chromaticity (Table S1). Subsequent statistical analyses were performed using average blackness, hue, saturation and brightness values per lizard and colour trait (ventral melanin-based spots, dorsal melanin-based spots and ventral carotenoid-based coloration).

Statistical analyses

Repeated measures ANOVAS (RM-ANOVA), using measurements taken before and after the experiment as the repeated measures, were used to analyse treatment effects on changes in coloration. Treatment effects on body condition were analysed by fitting a RM-ANCOVA with body mass as dependent variable and body size (SVL) as covariate. A two-factor ANOVA was used to analyse the effects of treatment on appetite. Initial models included corticosterone treatment, feeding regimen and their interaction as fixed factors. Final models were obtained by dropping nonsignificant three-way interactions between corticosterone treatment, feeding regimen and time. The normality and homoscedasticity of the residuals of all models were verified. Log- and power transformations were used when the assumptions were not fulfilled, and permutational RM-ANOVAS based on 9999 permutations were used in those cases where transformations did not solve distributional problems (Vallejo *et al.*, 2010). *Post hoc* comparisons were performed using paired *t*-tests (Looney & Stanley, 1989), which were based on 9999 permutations in permutational RM-ANOVAS. To control for the family-wise Type I error rate, the P values of *post hoc* tests were adjusted using the Bonferroni correction. Three lizards died during the experiment (one from the high-fed and corticosterone control group, one from the low-fed corticosterone group and one from the low-fed corticosterone control group) and were excluded from the analyses. No differences existed between treatment groups in body size (SVL), body mass and body condition at the beginning of the experiment (all $P > 0.32$). Coloration did not differ between treatments at the onset of the experiment (all $P > 0.12$), with the exception of saturation of the carotenoid-based coloration, which was higher in the high-fed group than in the low-fed group ($F_{1,30} = 9.30$, $P = 0.005$). No differences existed between corticosterone treatment groups for this trait ($F_{1,30} = 2.35$, $P = 0.136$). Analyses were conducted using JMP 8.0.2 (SAS Institute, Cary, NC, USA) and R 2.14.2 software (R Foundation for Statistical Computing, Vienna, Austria).

Results

Effects on body condition and appetite

Food availability significantly affected body condition (ANCOVA with body mass and SVL as covariate: feeding regimen × time interaction: $F_{1,29} = 43.41$, $P < 0.001$). In the high-fed group, body condition increased on average (± SE) by 12.13 ± 2.04% (*post hoc t*-test: $t_{16} = 5.99$, $P < 0.001$) over the course of the experiment, whereas no significant change was observed in the low-fed group (*post hoc t*-test: $t_{15} = 2.03$, $P = 0.12$). No significant effect of corticosterone treatment was observed on body condition, and no significant interaction was observed between corticosterone and feeding regimen ($F_{1,29} < 0.01$, $P = 0.99$, and $F_{1,29} = 0.62$, $P = 0.44$, respectively). On average, lizards ate 71 ± 4% of the offered larvae. This proportion did not significantly differ between groups, indicating no differences in appetite (feeding regimen: $F_{1,30} = 0.72$, $P = 0.40$, corticosterone treatment: $F_{1,30} = 0.55$, $P = 0.47$; interaction: $F_{1,29} = 1.85$, $P = 0.18$).

Effects on coloration

Corticosterone treatment significantly affected the blackness of the ventral melanin-based spots (corticosterone treatment × time interaction, Table 1 and Fig. 1). Over the course of the experiment, blackness significantly decreased in corticosterone-treated lizards, whereas no significant change was observed in control lizards. Corticosterone treatment also significantly affected the hue and saturation (corticosterone treatment × time interaction, Table 1). Over the course of the experiment, hue significantly decreased in control lizards (i.e. became redder), whereas no significant change was observed in corticosterone-treated lizards (Fig. 1). Saturation significantly decreased in corticosterone-treated lizards (i.e. exhibited greyer spots), with no significant change in the control group (Fig. 1). Brightness increased (i.e. spots became less dark) over the course of the experiment (time effect in Table 1), but this change was unaffected by corticosterone treatment, feeding regimen or their interaction (Table 1).

Over the course of the experiment, the dorsal melanin-based spots became brighter and less black (time effect in Table 1; Fig. 2). This change was unaffected by corticosterone treatment, feeding regimen or their interaction. No significant time or treatment effects were observed for hue and saturation (Table 1; Fig. 2).

Blackness of the carotenoid-based coloration significantly increased throughout the experiment, but was unaffected by any of the treatments (Table 1, Fig. 3). Corticosterone treatment significantly affected the hue of the ventral carotenoid-based coloration (corticosterone treatment × time interaction, Table 1 and Fig. 3). Hue significantly decreased (became more orange) in the control group, whereas no significant change was observed in corticosterone-treated lizards (Fig. 3). Neither feeding regimen nor the interaction between feeding regimen and corticosterone treatment significantly affected hue (Table 1). Saturation of the ventral carotenoid-based coloration significantly decreased during the experiment (change [mean ± SE]: −9.5 ± 1.8%; time effect in Table 1). However, this change was unaffected by corticosterone treatment, feeding regimen or their interaction (Table 1). Brightness of the carotenoid-based coloration was significantly affected by corti-

Table 1 Effects of corticosterone treatment and feeding regimen on ventral and dorsal melanin-based spot coloration and on the carotenoid-based coloration in male common lizards. Statistics represent the within-subject effects from RM-ANOVAS. For all colour variables, three-way interactions between corticosterone treatment, feeding regimen and time were not significant (see below) and removed from the final models.

	Blackness (%)			Hue (°)			Saturation (%)			Brightness (%)		
	F	d.f.	*P*	*F*	d.f.	*P*	*F*	d.f.	*P*	*F*	d.f.	*P*
Ventral melanin-based spot coloration												
Time	2.88	1,30	0.100	1.27	1,30	0.268	25.09	1,30	<0.001	7.12	1,30	0.012
Feeding regimen × time	0.04	1,30	0.845	0.21	1,30	0.652	0.33	1,30	0.570	0.75	1,30	0.956
Corticosterone treatment × time	7.38	1,30	0.018	13.14	1,30	0.001	13.92	1,30	<0.001	<0.01	1,30	0.998
Interaction × time	3.47	1,29	0.073	1.24	1,29	0.241	2.52	1,29	0.124	3.49	1,29	0.072
Dorsal melanin-based spot coloration												
Time	15.86	1,30	<0.001	0.57	1,30	0.476*	1.63	1,30	0.211	16.84	1,30	<0.001
Feeding regimen × time	<0.01	1,30	0.958	0.04	1,30	0.857*	0.28	1,30	0.599	<0.01	1,30	0.976
Corticosterone treatment × time	0.11	1,30	0.738	3.89	1,30	0.066*	1.76	1,30	0.195	0.34	1,30	0.564
Interaction × time	0.91	1,29	0.347	4.49	1,29	0.120*	0.22	1,29	0.197	0.60	1,29	0.445
Ventral carotenoid-based coloration												
Time	68.85	1,30	<0.001	0.45	1,30	0.506*	13.85	1,30	<0.001	97.20	1,30	<0.001
Feeding regimen × time	0.11	1,30	0.738	<0.01	1,30	0.938*	0.51	1,30	0.481	0.25	1,30	0.619
Corticosterone treatment × time	1.75	1,30	0.196	4.00	1,30	0.047*	1.72	1,30	0.199	6.68	1,30	0.015
Interaction × time	3.73	1,29	0.063	1.78	1,29	0.197*	0.24	1,29	0.624	1.41	1,29	0.244

**P* values from a permutational RM-ANOVA.

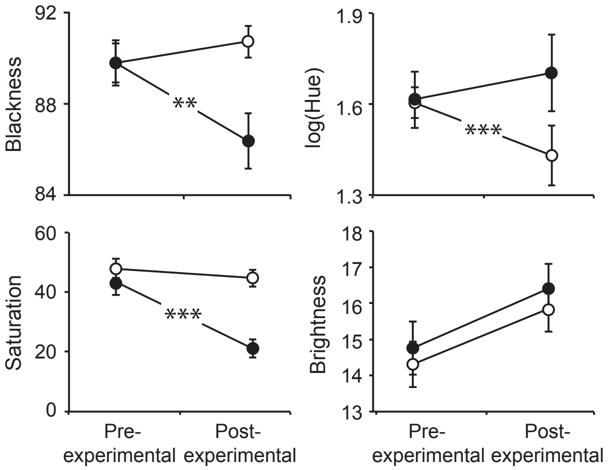

Fig. 1 Effects of corticosterone treatment on ventral melanin-based spot coloration of male common lizards. Data represent pre- and post-experimental means (± SE) of blackness (%), hue (°), saturation (%) and brightness (%) in corticosterone-treated (filled circles) and control lizards (empty circles). Asterisks indicate a significant interaction between time and corticosterone treatment in post hoc tests (** $P \leq 0.01$, *** $P \leq 0.001$).

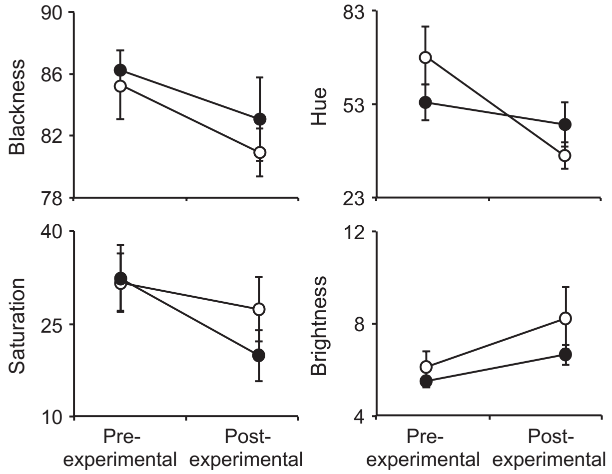

Fig. 2 Effects of corticosterone treatment on dorsal melanin-based spot coloration of male common lizards. Data represent pre- and post-experimental means (± SE) of blackness (%), hue (°), saturation (%) and brightness (%) in corticosterone-treated (filled circles) and control lizards (empty circles).

costerone treatment, but not by feeding regimen or the interaction between the two factors (Table 1; Fig. 3). A significant decrease in brightness was observed in both corticosterone-treated and control lizards, but this effect was significantly greater in corticosterone-treated lizards (corticosterone treatment × time interaction in Table 1 and Fig. 3).

Discussion

Corticosterone negatively affected different measures of the ventral carotenoid- and melanin-based coloration of male common lizards, whereas food availability had no effect on coloration despite significantly affecting body condition. Corticosterone prevented the increase in redness (i.e. decrease in hue) observed in the melanin-

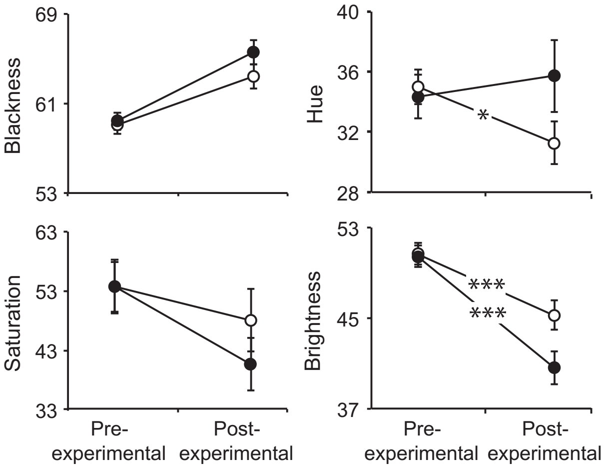

Fig. 3 Effects of corticosterone treatment on the ventral carotenoid-based coloration of male common lizards. Data represent pre- and post-experimental means (± SE) of blackness (%), hue (°), saturation (%) and brightness (%) observed in corticosterone-treated (filled circles) and control lizards (empty circles). Asterisks indicate a significant interaction between time and corticosterone treatment in post hoc tests (*$P \leq 0.05$, ***$P \leq 0.001$).

and carotenoid-based coloration of control lizards. Additionally, corticosterone reduced the blackness and saturation of the ventral melanin-based spots, and it negatively affected the brightness of the carotenoid-based coloration. These results therefore show that melanin- and carotenoid-based ornaments of male common lizards are phenotypically plastic, and they contrast with the ubiquitous belief that melanin-based traits are under smaller environmental but tighter genetic control than other colour traits (Badyaev & Hill, 2000). Moreover, our findings support the hypothesis that corticosterone regulates the expression of the different types of colour traits that can be simultaneously displayed by individuals, which further suggests that the evolution of different types of colorations may be governed by similar evolutionary mechanisms.

The absence of an effect of food availability and body condition on carotenoid-based coloration is in line with previous findings in the common lizard and in the brown anole, *Norops sagrei,* showing that an experimental decrease in body mass via manipulation of food availability does not affect the expression of carotenoid-based coloration (Cote *et al.*, 2010; Steffen *et al.*, 2010). These findings contrast with studies in birds, which have shown that food availability substantially determines the expression of carotenoid-based ornaments (Hill, 1994; McGraw *et al.*, 2005). However, in contrast to birds, carotenoid-based coloration is not affected by carotenoid ingestion in the common lizard, in the brown anole or in the other lizard species studied to date (Olsson *et al.*, 2008, 2013; Steffen *et al.*, 2010; San-Jose *et al.*, 2012b). This suggests that lizard coloration may not be carotenoid-limited, which could explain why food availability did not affect carotenoid-based ornaments. Similarly, our study supports that the melanin-based coloration of male common lizards is not linked to food availability. In this sense, our study does not support previous studies suggesting that the amount of certain aminoacidic precursors in the diet limits melanin synthesis and melanin-based colorations (Stoehr, 2006; McGraw, 2008).

Despite the absence of a link between ventral coloration and body condition, the observed corticosterone effect on ventral coloration supports that this stress hormone mediates condition dependence of both melanin- and carotenoid-based ornaments in the common lizard. This finding is partly in line with previous studies in the barn owl, *Tyto alba* (Roulin *et al.*, 1998, 2008), showing that corticosterone but not body condition mediates the condition-dependent component of the melanin-based coloration. Here, we show that this effect is extensive to different types of ornaments, showing that melanin- and carotenoid-based ornaments plastically respond to changes in corticosterone levels but not in body condition. Animal condition is complexly determined by different factors besides body condition or related physiological aspects such as energy or resource stores (Hill, 2011). Other vital processes such as oxidative stress influence health and, thereby, condition and have been shown to affect colour traits with condition-dependent expression (Monaghan *et al.*, 2009; Metcalfe & Alonso-Alvarez, 2010). In the common lizard, high corticosterone levels increase oxidative damage (Cote *et al.*, 2010), suggesting that high corticosterone levels can affect condition and that the ventral melanin- and carotenoid-based coloration of male common lizards function as an honest signal of male condition. Moreover, our results show that corticosterone treatment affected ventral coloration but not that of the dorsal region (i.e. dorsal melanin-based spots), which is subjected to natural selection (Lepetz *et al.*, 2009). This shows that corticosterone specifically mediates the condition dependence of ventral ornaments and that the effects observed on these traits do not result from general, unspecific effects of corticosterone or, because the expression of the overall coloration (ornamental and nonornamental) is condition dependent to some extent (Cotton *et al.*, 2004).

Different nonexclusive hypotheses might explain how corticosterone mediated general colour changes in both melanin- and carotenoid-based ornaments. The corticosterone effect on melanin-based coloration may stem from its action at specific glucocorticoid receptors in melanophores, leading to an aggregation of melanosomes within melanophores and/or a decrease in melanin concentration (Greenberg, 2002; Ducrest *et al.*, 2008; Roulin *et al.*, 2008). Like melanophores, iridophores are modulated by cell receptors that belong to the same receptor superfamily as glucocorticoids receptors (Evans, 1988; Miwa & Yamano, 1999). As colour change in carotenoid-based coloration of common lizards is mediated by iridophores rather than carotenoids (San-Jose *et al.*, 2013), corticosterone may modulate carotenoid-based coloration via iridophore cell receptors, which would explain why elevated blood corticosterone levels impaired both carotenoid- and melanin-based ventral coloration. On the other hand, corticosterone may indirectly influence carotenoid- and melanin-based coloration by increasing oxidative stress (Costantini *et al.*, 2008, 2011), as proposed for male common lizards (Cote *et al.*, 2010; San-Jose *et al.*, 2012b) and demonstrated for other species (Galvan & Alonso-Alvarez, 2008), respectively.

Our results contrast with previous findings in the common lizard, showing that corticosterone increases redness of male ventral carotenoid-based coloration (Fitze *et al.*, 2009; San-Jose *et al.*, 2013). Positive effects of stress hormones on coloration have also been reported in zebra finches, *Taeniopygia guttata* (McGraw *et al.*, 2011), indicating that such effects are not rare or specific to the common lizard and that there exists a complex link between corticosterone and coloration in animals. The main difference between this and the previous studies on the common lizard (particularly between this study and San-Jose *et al.*, 2013; which was performed in the same laboratory, year and lizard population and using the same corticosterone dose) was the duration of the corticosterone administration, which was one to 2 weeks longer in this study than in previous studies. Differential corticosterone effects on coloration in relation to treatment duration have not been investigated yet, but treatment duration is known to modulate corticosterone effects on other traits (Nelson, 2005).

Additionally, in contrast to Cote *et al.* (2010), who found that corticosterone affects coloration and body mass of well-fed but not of food-restricted lizards, we found no food-dependent corticosterone effects on coloration or body mass. The difference between the two studies may be explained by differences in treatment duration or by differences in the applied feeding treatments. Given that the prey items used here and in Cote *et al.'s* study (2010) may have differed in nutritional quality and that the ventral coloration of male common lizards has been shown to vary according to the amount of certain dietary nutrients (San-Jose *et al.*, 2012b, 2013), the absence of this effect suggests that the corticosterone effect may be modulated by food quality rather than food quantity. In contrast to Cote *et al.* (2010), we found that body mass significantly increased in well-fed lizards, which suggests that we may have provided lizards with more food resources than Cote *et al.* (2010). The fact that no negative trends in body mass change were found during this experiment (not even in the low-fed lizards, whose body mass showed no significant changes) and that the here-provided prey items (*Galleria mellonella*) are richer in energy content (fats) (Finke, 2002) than the prey items used in other studies support this hypothesis. Nevertheless, additional studies are needed to understand the origin of the detected differences between this and previous studies and thus of the factors modulating the action of corticosterone.

In summary, our findings reveal that blood corticosterone levels negatively affect multiple colour ornaments in the common lizard, which is in line with condition dependence of both signals. Previous studies in the common lizard showed that females are less interested in males with higher corticosterone levels (Gonzalez-Jimena & Fitze, 2012), supporting that stress and corticosterone levels play an important role in sexual selection. Corticosterone regulation of multiple ornaments may be common in animals given that corticosterone effects on single colour traits have been demonstrated in several different species (Loiseau *et al.*, 2008; Roulin *et al.*, 2008; Fitze *et al.*, 2009), and given that the mechanisms by which stress hormones act are quite conserved across taxa (e.g. Davis *et al.*, 2008). Moreover, corticosterone could affect other traits and mediate condition dependence of other visual (e.g. behavioural displays), chemical or acoustic signals (Wingfield & Silverin, 1986; Aragón *et al.*, 2008). As shown here, stress and/or stress hormones such as corticosterone mediate condition dependence of different types of ornaments, inducing covariation among multiple ornaments (i.e. males with low corticosterone levels exhibit more intense melanin- and carotenoid-based coloration). Such patterns of covariation further indicate that multiple ornaments may not evolve independently as they may be subject to correlational selection (Endler, 1995), which may help to understand why multiple signalling is so common in nature (Hebets & Papaj, 2005).

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Supporting information

Additional Supporting Information may be found in the online version of this article:
Table S1 Pairwise correlations among blackness, hue, saturation, and brightness.

Data deposted at Dryad: doi:10.5061/dryad.km25k

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